The University of Chicago

THE PHARMACOLOGY AND ANATOMY OF THE HYPOPHYSIS OF THE ARMADILLO

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY
1938

By
FRANCES K. OLDHAM

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THE PHARMACOLOGY AND ANATOMY OF THE HYPOPHYSIS OF THE ARMADILLO [1,2]

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TWO PLATES (FOUR FIGURES)

INTRODUCTION

This report contains an embryological, anatomical and pharmacological analysis of the hypophysis of the nine-banded armadillo (Dasypus novemcinctus) with particular reference to the site of elaboration of the neural lobe hormones.[2,3] This question has been the center of a lively controversy since Oliver and Schäfer (1895) first demonstrated pharmacological activity in extracts of the gland. As secretory granules were not found in the cells of the pars nervosa, investigators were long unwilling to ascribe a secretory role to the neuroglia-like cells, which are the only cells regularly found in significant numbers in this portion of the gland. The association between the lobes is frequently so intimate that many workers, especially in the clinical fields, believe that the oxytocic and pressor

[1] Submitted in partial fulfillment of the requirements for the degree of doctor of philosophy at The University of Chicago. Acknowledgment is made of a grant from the Rockefeller Foundation which made this work possible.

[2] A preliminary report of this work was given at the April, 1938 meeting of the American Society for Pharmacology and Experimental Therapeutics.

[3] The following terminology will be used. The derivatives of the buccal ectoderm and of the neural ectoderm will be referred to as the pars buccalis and the pars nervosa respectively. The pars buccalis consists of the anterior lobe, pars tuberalis and pars intermedia (intermediate lobe). The pars nervosa consists of the neural lobe and the neural stalk. The term 'stalk' refers to that area between the neural lobe and the brain which was removed for pharmacological assay. This portion includes the neural stalk, the pars tuberalis and a variable amount of brain.

hormones are formed, along with the melanophore-dispersing hormone, by the glandular cells of the pars intermedia or from their degradation products, and diffuse into the pars nervosa, appearing there in the form of colloid droplets, the so-called Herring bodies. This view is summarized by Rolleston ('36).

In the past few years, studies along widely divergent lines have indicated that the oxytocic, pressor and anti-diuretic hormones are formed by the pars nervosa. The tissue culture experiments of Geiling and Lewis ('35) showed that growths of the pars intermedia of the rat and mouse possess melanophore-dispersing, but no pressor activity. Cultures of neural lobe admixed with a small amount of pars intermedia had both pressor and melanophore-dispersing activity. Similarly, Anderson and Haymaker ('35) found melanophore-dispersing but no oxytocic or anti-diuretic activities in intermediate lobe growths.

Fisher, Ingram and Ranson ('38) in an extensive series of experiments produced diabetes insipidus in cats by means of lesions in the supraoptico-hypophyseal tracts and found that the hypophyses of these animals showed pronounced atrophic changes accompanied by a loss of oxytocic and pressor activity. The melanophore-dispersing activity was, however, unaffected and the intermediate and anterior lobes were unaltered histologically.

Gersh and Tarr ('35) using the Altmann-Gersh freezing-drying technique concluded that the Herring bodies are fixation artefacts arising from the evenly distributed albumin-like protein substance which infiltrates the pars nervosa. By comparison of the extractibility of this material and of the pressor hormone, they claimed the two are unrelated.

The most convincing proof of the role of the pars nervosa in the elaboration of the oxytocic, pressor and anti-diuretic hormones was advanced by Geiling and his associates (DeLawder, Tarr and Geiling, '34; Geiling, '35; Wislocki and Geiling, '36) through their investigations of the hypophysis of the whale and the chicken and more recently on the manatee

(Oldham, McCleery and Geiling, '38). They showed that although in these species an anatomically discrete pars intermedia is lacking and cells of the pars intermedia type are absent from the pars nervosa, pressor and oxytocic hormones are present in neural lobe extracts. The melanophore-dispersing hormone, on the other hand, is present only in anterior lobe extracts.[4] Since the anterior and neural lobes are clearly separated by connective tissue, passage of the hormones from one lobe to the other is improbable. Furthermore, the studies of Wislocki and King ('36) and Wislocki ('37 a, b) on the blood supply of the hypophysis show that the anterior and neural lobes have an independent blood supply and that venous drainage is such that interchange of secretions by this route is unlikely. The lymphatic drainage of the several parts of the hypophysis does not seem to have been worked out.

While the hypophyses of the whale and the chicken have furnished interesting results, they have certain disadvantages for experimental purposes. The small gland in the chicken is buried in a deep sella turcica and is difficult to approach. For unavoidable reasons, the whale pituitary could not be removed until 8 to 24 hours after the death of the animal, so that post-mortem changes were inevitable. Search was made, therefore, for another species in which an intermediate lobe was not present. A preliminary survey by M. R. Lewis of the Carnegie Institution of Embryology showed that the armadillo hypophysis answered this description, and with her acquiescence a detailed study of the pharmacology and anatomy of this gland was undertaken.[5]

[4] Three or four active substances can be demonstrated in commercial extracts of the posterior pituitary, namely the pressor (anti-diuretic), oxytocic and melanophore-dispersing hormones. At present it is generally agreed that the pressor and anti-diuretic activities are mediated by the same hormone. Furthermore, it has been conclusively shown that the melanophore dispersing hormone arises in the pars buccalis. Accordingly in this paper, the term 'neural lobe hormones' refers to the pressor (anti-diuretic) and oxytocic hormones.

[5] During the course of preparation of this manuscript, there appeared a paper by Wislocki ('38) containing a description of the topography of the armadillo hypophysis. His findings accord well with the more detailed observations and experiments reported here.

The armadillo proved to be an ideal animal for this purpose. The animals are relatively inexpensive and can be obtained at short notice any time of the year; they can be kept in captivity for short lengths of time. The hypophysis is comparatively large and the lobes are easily approached and separated.

ANATOMY OF THE ARMADILLO HYPOPHYSIS

Material and methods

The armadillo is a member of the Edentata (Zenarthra), one of the lower orders of mammals. The animals used in this study (Dasypus novemcinctus) came from Texas. Glands were obtained from males and from pregnant and non-pregnant females as well as from embryos. While the age of the adult animals was not known, two of them were quite young, since the sphenoid bone was incompletely ossified. Embryos of the following crown-rump were obtained: 3.4 cm., 4.5 cm., 5 cm., 5.8 cm. and 7.7 cm. and a newly born animal of about 10 cm. Although the early stages are missing from this series, the material on hand permits an investigation of the fate of Rathke's pouch and the general development of the gland.

The armadillos were killed with ether, and the glands fixed immediately in formol-Zenker. A small amount of hypothalamus was left attached to the infundibular stalk in order to ensure complete removal of the pars tuberalis. In several cases, a portion of the underlying bone was removed together with the gland. After fixation, the material was embedded in nitrocellulose, serially sectioned at 8 μ and stained by the hematoxylin-eosin-azure II and the Mallory-azan methods. The glands were cut in either the sagittal, horizontal or frontal planes. Decalcification, when necessary, was done with 3% HNO_3 subsequent to embedding. Certain other histological methods were used. Bodian's ('36) colloidal silver method was used as a nerve fiber stain. This was not successful when tried on nitrocellulose-embedded material. Satisfactory results were obtained, however, when paraffin was used. Penfield's ('28) modification of the del Rio Hortega silver carbonate stain was used for impregnating the neuroglia cells of

the pars nervosa. One gland was fixed in alcohol and stained with thionin blue for mast cells. Twenty-two glands were used for histological study and twenty-one for pharmacological assay.

Gross anatomical observations

The armadillo hypophysis is a roughly oval structure lying in a shallow depression on the superior surface of the sphenoid bone. The long axis of the gland lies transversely to that of the body and measures approximately 0.9 cm. The antero-posterior diameter is approximately 0.6 cm. In most instances, the meninges over the neural lobe are dark because of the

TABLE 1

Weight in milligrams of the separate portions of the hypophysis

	APRIL, 1936 [1]			JANUARY, 1937 [2]		
	Ant. lobe	Post. lobe	Stalk	Ant. lobe	Post. lobe	Stalk
	40.30	9.46	6.20	46.00	8.25	...
	44.90	9.00	1.90	22.50	7.00	...
	36.40	6.64	3.51	38.00	8.00	2.85
	36.50	6.94	4.10	29.00	8.30	4.11
	34.50	8.70	5.30	24.00	8.90	0.90
Total	192.60	40.74	21.01	159.50	40.45	7.86
Average	38.52	8.15	4.20	31.90	8.09	2.62

[1] Sex not recorded.

[2] Male animals.

melanophores in them. In the fresh condition the somewhat triangular neural lobe can be distinguished by its very white appearance. It occupies an excavation in the posterior and dorsal surfaces of the gray-tan anterior lobe. The neural lobe is attached to the neural stalk which extends anteriorly and dorsally to join the hypothalamus. On either side of the junction of the infundibular stalk and the neural lobe, projections of anterior lobe tissue extend partially around each side of the pars nervosa and nearly meet in the midline of its dorsal surface. The weights of the two lobes of twenty-one armadillos are recorded in tables 1 and 2. The anterior lobe is roughly four times as heavy as the neural lobe.

The gland is embedded in a fairly thick dural sheath. An extension of this dense connective tissue completely separates the two lobes. An obvious subdural space is not apparent. The connections between this connective tissue and the lobes are fairly loose and the neural lobe can be lifted away from the anterior lobe by gross dissection. This is facilitated by the use of a low power dissecting microscope. Neither a pars tuberalis nor a pars intermedia is visible on gross inspection.

TABLE 2

Weight in milligrams of the separate portions of the hypophysis

	WEIGHT OF SEPARATED PORTIONS IN MILLIGRAMS			BODY WEIGHT IN KILOGRAMS
	Ant. lobe	Post. lobe	Stalk	
November, 1937 [1]	43.40	6.41	3.76	4.45
November, 1937 [1]	29.40	8.55	0.68	3.37
November, 1937 [1]	16.80	4.52	...	1.72
November, 1937 [1]	34.60	10.10	4.78	4.00
November, 1937 [1]	24.40	8.72	4.46	4.20
November, 1937 [1]	31.40	10.10	3.50	3.90
November, 1937 [1]	34.40	8.20	4.65	2.62
November, 1937 [1]	43.80	11.60	...	3.90
Total	258.20	68.20	21.83	28.16
Average	32.27	8.52	3.64	3.52
February, 1938 [2]	25.50	7.96	10.90	2.97
February, 1938 [2]	37.90	7.51	...	3.90
February, 1938 [2]	43.70	8.16	7.51	3.45
Total	107.10	23.63	18.41	10.32
Average	35.70	7.88	9.21	3.44

[1] Female animals killed by electrocution.

[2] Female animals.

Histological findings

General observations. The distinctive microscopic feature of the armadillo hypophysis is the absence of a discrete pars intermedia. The anterior and neural lobes are completely separated by connective tissue and no cells of the type found in the pars intermedia of most animals can be seen in the neural lobe. The clear-cut separation of the two lobes is particularly well seen in sections stained with the Mallory-azan

method. In them, a layer of dense collagenous connective tissue of variable thickness is apparent as a deeply blue staining septum.

Anterior lobe. The anterior lobe of the armadillo hypophysis consists of nests of closely packed chromophobe and chromophil cells supported by a network of reticular connective tissue. Posteriorly the meshes of the connective tissue are finer and the cells are more closely packed. Numerous irregular blood sinuses are present in this connective tissue. A drop of colloid may be seen frequently in the center of a nest of cells. The relative number and the distribution of the various types of anterior lobe cells varies widely in the different glands and in different regions of the same gland.

The predominant cell in the anterior lobe of the armadillo hypophysis is the alpha cell. With the hematoxylin-eosin-azure II stain, the cytoplasm of these cells contains a varying number of small eosinophilic granules with frequently a lighter staining area to one side of the nucleus. In the Mallory-azan preparations the granules of some of the cells stain with azo-carmine while in others they stain with orange-G. The spherical nucleus contains a central nucleolus and a varying number of large chromatin particles at the pheriphery and finer ones scattered through the nucleus. In some of these cells, the nucleus stains darkly and is less regular in outline.

The beta cells are scarce in the armadillo hypophysis. With hematoxylin-eosin-azure II, the cytoplasm contains pale blue granules. Since the granules are very fine, the beta cells are hard to distinguish from the chromophobes with this stain. With the Mallory-azan stain, the granules are a more intense blue. The cytoplasm is frequently vacuolated and the irregularly-shaped nuclei stain darkly and appear pycnotic. The negative image of the Golgi apparatus is usually clearly seen in the Mallory-azan preparations.

Two main types of chromophobic cells can be distinguished. One type is found scattered among the chromophilic cells. The non-granular cytoplasm is usually small in amount and

stains a faint blue with hematoxylin-eosin-azure II. The nuclei are similar to those of the alpha cells. The second type of chromophobe is usually found in nests. The nucleus appears shrivelled and stains intensely. Faint basophilic strands divide the cytoplasm into compartments which have the appearance of large vacuoles. A number of forms intermediate between chromophils and chromophobes may be seen.

An unusual type of cell was seen in the anterior lobe of all the armadillo hypophyses examined. These cells are easily distinguished by their size and staining properties from the chromophobe and chromophil glandular cells and from the connective tissue elements. Their diameter is from one and a half to three times that of the acidophils. At first glance they are somewhat suggestive of sympathetic ganglion cells. Neurofibrils have not been demonstrated in them; occasionally the cytoplasm contains large basophilic particles which stain intensely with the hematoxylin-eosin-azure II and are somewhat suggestive of irregular Nissl bodies. The peripheral cytoplasm is usually clear and the cell limits hard to define. A layer of cytoplasm staining a faint blue with hematoxylin-eosin-azure II surrounds the nucleus and extends irregularly into the cytoplasm. The diameter of the large nucleus is about twice that of the average glandular cell. It is usually situated at one side of the cell and may be round or oval in outline and is occasionally slightly indented. There is a single prominent nucleolus (or rarely two or more), irregular in outline and usually eccentric in position. In many of the cells, small particles of chromatin are scattered throughout the nucleus. In others, there are finely granular linin strands with an occasional chromatin particle. These cells are found mainly on each side of the mid-line in the anterior two-thirds of the anterior lobe and usually in its dorsal half. They are most numerous in the region where the anterior lobe and the pars tuberalis are in contact. They are found there in small groups and the individual cells are often partially surrounded by connective tissue. More anteriorly in the anterior lobe the cells are in general smaller and less conspicuous.

Colloid-filled clefts are seen in the posterior portion of the anterior lobe. These are seen best in frontal and horizontal sections and may be quite extensive. They are usually lined by a flattened epithelium, although occasionally by a ciliated, cuboidal epithelium. The cells between these clefts and the juxta-neural border of the anterior lobe are similar to those found elsewhere in the lobe.

Pars tuberalis. The extensive pars tuberalis consists of a sheet of cells several layers in thickness surrounding the stalk and extending under the hypothalamus both anteriorly and posteriorly, the anterior extension being the more prominent. Most of the cells are chromophobes, although occasionally granular acidophils are present. The cells are arranged characteristically in small follicles in the center of which are colloid droplets. There is an extensive and well-vascularized connective tissue framework which permeates the lobe and extends as a continuous layer separating the pars tuberalis from the neural lobe. Ventrally, the transition between the anterior lobe and the pars tuberalis is a gradual one and the two cannot be separated.

Neural lobe. The neural lobe of the armadillo hypophysis contains more cells and more Herring bodies than are seen in most other species. The blood supply is rich and there is much perivascular connective tissue which in sections is made prominent by the Mallory-azan stain. The infundibular recess extends down the stalk; isolated portions in the form of ependymal lined spaces are often visible in the neural lobe. These isolated portions, when traced through serial sections, are found to be independent of the main cavity. Preparations stained by Bodian's method for nerve fibers show numerous fibers streaming down from the hypothalamus and branching extensively in the neural lobe. The exact nature of these fibers and of their terminations in the armadillo remains to be determined.

The most common cell type in the neural lobe has a small irregularly-shaped and deeply stained nucleus containing several acidophil nucleoli. With the ordinary techniques, the

long cytoplasmic processes of these cells cannot always be distinguished from the somewhat fibrillar background of the lobe. With Penfield's silver carbonate stain, these processes are made more prominent. Several processes, often with numerous branches, may arise from a single cell; in many cases they are in close contact and often seem to be continuous. They frequently end on blood vessels or on the connective tissue capsule of the lobe.

Another type of cell is present in considerable numbers and is conspicuous because of its large size. It is most frequently seen in the anterior and central portion of the lobe. These cells appear usually in groups and, occasionally, cytoplasmic membranes cannot be made out between the individual cells so that they appear multi-nucleated. The diameter of the nuclei of these cells is two to three times that of the length of the nuclei of the neuroglia-like cells. The nuclei of the large cells contain one or more prominent, relatively large nucleoli and a scattering of fine chromatin particles. With the hematoxylin-eosin-azure II stain, a fairly extensive, faintly or deeply basophilic and finely granular cytoplasm can be seen. There are numerous intermediate cells between the large and the neuroglia-like cells. In the connective tissue of the neural lobe, fibroblasts are common and are not readily distinguished from many of the neuroglia-like cells. Mast cells and plasma cells are present in small numbers.

Both melanoblasts and melanophores are seen in the majority of the glands. These cells appear to be confined to the meninges and enter the neural lobe only in association with blood vessels. They are seen in greatest numbers in the leptomeninges of the ventral surface of the hypothalamus and in the connective tissue of the pars tuberalis. Another common site is over the dorsal surface of the neural lobe and to a lesser extent of the anterior lobe. They are also present in the connective tissue separating the two lobes and occasionally in the connective tissue network of the periphery of the anterior lobe.

Neural stalk. The neural stalk is very vascular. The ependymal-lined infundibular recess occupies the central portion. Neuroglia-like cells and cells with large nuclei similar to those in the neural lobe are found to the side of the recess. Bodian's stain for nerve fibers shows fibers streaming down on either side of the recess.

Embryological findings

The anterior lobe of the 3.4 armadillo embryo consists of loosely packed cells, separated by strands of connective tissue and large vascular sinuses. The majority of the cells are chromophobes, others have a faintly-staining non-granular cytoplasm. Projections of anterior lobe tissue extend posteriorly and partially surround the neural stalk. A large cleft occupies the posterior portion of the lobe, and also extends into the posteromedial projections of the lobe. The posterior border of this cleft is composed of several irregularly arranged layers of closely packed cells. The anterior border of the cleft is formed mostly by cell nests.

The neural lobe lies posterior to the anterior and is separated from the latter by a layer of collagenous connective tissue. The cells are seen in groups in the central portion of the lobe and are of two types. In one the nucleus is relatively small, roughly oval and stains fairly deeply. The nucleus of the other type is larger, vesicular and usually spherical. The neural lobe is connected with the brain by the neural stalk, which contains the ependymal-lined infundibular recess.

The hypophyses of the 4.5- and the 4.8-cm. embryos are similar. The cell nests in the anterior lobe are more closely packed than in the 3.4-cm. embryo. The more deeply staining cells now appear to be the predominant type, although chromophobes are also abundant. The cleft is still conspicuous. The cells composing its posterior border seem to be less closely packed, and the chromophobic cytoplasm can be seen in many of the cells.

In the neural lobe many of the cells with the small nuclei appear to be migrating toward the periphery of the lobe.

Long cytoplasmic processes extend from these cells. The cells with the larger, more vesicular nuclei tend to remain in the center of the lobe.

The 5-cm. and the 5.8-cm. embryos are essentially the same. There is a considerable increase in the size of the anterior lobe both laterally and anteriorly. Occasional faintly granular alpha cells are seen for the first time in the anterior lobe. There is a great increase in the number of cells in the neural lobe, specially those with the small nuclei. Mitoses are frequent. Melanophores are seen in the connective tissue surrounding the hypophysis of the 5-cm. embryo.

The hypophysis of the 7.7-cm. embryo approaches closely the form of the adult gland. The anterior lobe is flattened dorsoventrally and the neural lobe lies on its dorsal and posterior surfaces. In mid-sagittal sections, only a small narrow cleft can be seen. More laterally, however, it is still fairly extensive.

In the newborn animal (approximately 10 cm.) the cleft is present as a narrow slit in the posterior and ventral portion of the gland. It is absent from the posterior extensions of the anterior lobe. Alpha cells are now numerous and deeply staining.

In all the embryos studied, the anterior and neural lobes are clearly separated by connective tissue and anterior lobe cells are never seen in the pars nervosa. Colloid droplets are not seen in the anterior lobe or Herring bodies in the neural lobe.

PHARMACOLOGY OF THE ARMADILLO HYPOPHYSIS

Material and methods

The glands were separated into three parts: anterior lobe, neural lobe and 'stalk.' The animals were anesthetized deeply with ether or stunned by electrocution. The skull was opened and the brain removed except for a small portion which was left attached to the neural stalk. The meningeal covering of the shallow sella was cut away, exposing the dorsal surface of the gland. The neural lobe was then lifted up and separated from the stalk. Care was taken to make this separation

sufficiently posterior to avoid inclusion of a portion of the pars tuberalis in the neural lobe. In spite of this precaution, serial sections of the separated portions of one gland show a few nests of pars tuberalis cells attached to the neural lobe. The neural stalk and the pars tuberalis which surround it, were removed along with a small portion of the brain attached to the stalk. Occasionally the connection between the neural stalk and the brain was accidentally severed and in these cases no brain tissue was removed. The connective tissue which separated the neural and anterior lobes was stripped aside and the anterior lobe was lifted away from the underlying connective tissue.

As the three portions were removed, they were weighed on a torsion balance and placed immediately into acetone. The weights of the individual portions are incorporated in tables 1 and 2. The variations in the weights of the 'stalk' can be readily explained by the impossibility of distinguishing this portion from the neural lobe on one hand, and the brain on the other, and because excess of brain tissue was usually included with the stalk in order to ensure complete removal of the pars tuberalis.

The separated and acetone-desiccated portions were then extracted with 0.25% acetic acid according to the specifications set forth in the United States Pharmacopoeia XI for the preparation of standard pituitary. Since the individual lobes are so small, it was found advisable to combine those of a small group of animals rather than to attempt to assay each gland separately. The extracts were assayed for pressor, anti-diuretic, oxytocic and melanophore-dispersing activities. All assays are expressed in terms of the international standard.

Pharmacological findings

Pressor hormone. The pressor activity was assayed by the blood-pressor raising effect on the phenobarbital-anesthetized cat, according to the recognized technique. The pressor activity of the armadillo neural lobe extracts was found to be equivalent to that of the standard powder (fig. 3). The ante-

rior lobe extracts have little or no pressor effect. Extracts of the 'stalk' contain considerably less activity than those from the neural lobe. However, they show a definite pressor effect, whereas the rise of blood pressure with the anterior lobe extracts is so slow and prolonged that it is apparently due to impurities or possibly to an accidental admixture with the active neural lobe.

Anti-diuretic hormone. The anti-diuretic activity was measured by a modification of Burn's ('31) technique, which in turn is an adaptation of Gibb's ('30) method. The results summarized in table 3 show that the anti-diuretic substance in the armadillo hypophysis is confined to the neural lobe and

TABLE 3

Comparison of anti-diuretic activity of the anterior and posterior lobes of the armadillo hypophysis

	CONTROL	STANDARD	POST. ARM.	ANT. ARM.
Group I	120.0	186.0	198.0	118.0
Group II	112.0	194.0	199.0	111.6
Group III	108.0	191.0	214.0	106.0
Group IV	97.5	202.5	213.0	124.0
Average	109.4	193.4	206.0	114.9

Each animal received 10 cc. of tap water at 37°C. intraperitoneally; and subcutaneously, 0.5 cc. of dilute pituitary solution (0.25 cc. of extract, 1 mg. per cubic centimeter, diluted with saline to 5 cc.).

that its activity in this species is approximately that of the standard power.

Oxytocic hormone. Assays by the standard technique employing the virgin guinea pig uterus show consistently that the oxytocic content of the neural lobe of the armadillo hypophysis is only about 10% that of the standard (fig. 4). Assays by the chicken blood pressure method (Coon, '38), however, show this activity to be about 30% that of the standard. Results by the two methods of assay regularly show such a discrepancy when there is a great preponderance of the pressor hormone (Coon, personal communication). Extracts of the anterior lobe and of the 'stalk' showed by both methods of assay, only traces of oxytocic activity.

Inactivation of the pressor and oxytocic hormones by alkali. In order to see whether any of the observed pressor or oxytocic effects of the armadillo hypophysis were due to impurities, the hormones were destroyed by alkali. To 0.5 cc. of neural extract was added 0.25 cc. 2 N NaOH and the mixture left overnight. It was then neutralized with 1 N HCl and the volume made up to 2 cc. with 0.25% acetic acid. Following this treatment, no pressor or oxytocic response could be elicited showing that the previous effects with unalkalinized preparations were due to the hormones and not to impurities.

TABLE 4

Melanophore activity of the anterior and neural lobes of the armadillo compared with the standard powder

TIME AFTER INJECTION (IN MINUTES)	ARM. POST. LOBE 0.3 MG.	ARM. POST. LOBE 0.06 MG.	ARM. ANT. LOBE 0.3 MG.	ARM. ANT. LOBE 0.03 MG.	ARM. ANT. LOBE 0.003 MG.	STANDARD POWDER 0.03 MG.	STANDARD POWDER 0.003 MG.
30	?	0	++	++	0	++	—
60	+	0	+++	++	?	++	++
120	?	0	+++	+++	0	+++	++
150	0	0	++	—	0	—	+
180	0	0	++	—	0	—	—
210	0	0	++	+	0	+	0
240	0	0	++	+	0	+	0

The extracts were diluted with 0.25% acetic acid so that 0.25 cc. contained the desired amount of material. Injection was made into the ventral lymph sac. Hypophysectomized frogs were used throughout. The degree of darkening of the frog is expressed as + (slight), ++ (moderate) and +++ (maximal).

Melanophore-dispersing hormone. Hypophysectomized frogs (Rana pipiens) were used for the assay of the melanophore-dispersing hormone, since it has been shown that in such animals the action of this hormone is apparently a specific one (Teague and Noojin, '38). These assays showed regularly the presence of the melanophore-dispersing hormone in the anterior lobe extracts with only traces in the neural lobe and 'stalk.' While this method of assay is not a very satisfactory one for quantitative results, nevertheless comparison of the activity of the anterior lobe preparations with that of the standard powder showed the latter to be about ten times stronger (table 4).

DISCUSSION

The distribution of the pressor, anti-diuretic, oxytocic and melanophore-dispersing hormones in the armadillo hypophysis indicates that the first three of these principles are formed in the pars neuralis and the last in the anterior lobe.

Various investigators (see summary by Atwell and Holley, '36) have shown that in many species, the intermediate lobe of the pars buccalis is responsible for the elaboration of the melanophore-dispersing substance. In most animals, the intermediate and neural lobes are in such intimate contact that complete separation of the two is impossible, so that the melanophore-dispersing hormone is found in posterior lobe extracts along with the pressor, anti-diuretic and oxytocic hormones. This led to the belief that these latter hormones might also arise in the intermediate lobe. In the armadillo hypophysis, however, there is no discrete intermediate lobe and cells of the type found in the intermediate lobe of other species are not present in the pars nervosa. Furthermore, the pars buccalis and the pars nervosa are clearly separated by connective tissue. In addition, assays of the separated lobes show clearly that the oxytocic, pressor and anti-diuretic hormones are present in the neural lobe extracts, while only traces of the melanophore-dispersing hormones are present in such extracts. On the other hand, the anterior lobe extracts are rich in melanophore-dispersing hormone but contain only traces of the oxytocic and pressor hormones, which are in all probability contaminants. Hence it appears that the neural lobe hormones are not elaborated by the cells that give rise to the melanophore-dispersing hormone, but are formed in the pars nervosa.

Absence of a pars intermedia has been previously reported in birds (de Beer, '26), the porpoise (Wislocki, '29), whale (Valso, '34; Geiling, '35; Wislocki and Geiling, '36) and manatee (Oldham, McCleery and Geiling, '38), but a satisfactory explanation for its absence in these species has not been advanced. Except in the birds, the embryonic development of the hypophysis has not been studied in any of these

species. Bruni ('14) and de Beer ('26) draw attention to the early closure of Rathke's pouch in the bird. However, in all the armadillo embryos examined, the pouch extends along the juxtaneural region of the anterior lobe and, in all cases, the anterior and neural lobes are closely associated although completely separated from one another by a layer of collagenous connective tissue. In this species, therefore, the absence of a discrete pars intermedia cannot be attributed to an early closure of Rathke's pouch.

It has been suggested that the formation of the intermediate lobe depends upon contact of the pars buccalis with the brain. Atwell and Holley ('36) extirpated the caudal region of the hypophyseal anlage in tadpoles and obtained silvery animals that underwent complete metamorphosis. Histological examination of the gland showed that the intermediate lobe was absent and that the anterior lobe was at some distance rostral to the neural lobe. These findings, supplemented by transplantation experiments (Atwell, '35), led to the suggestion that contact of the epithelial anlage with the brain floor may be necessary for the early development of the intermediate lobe. Gaillard ('37) grew explants of the anterior and posterior lobes in tissue culture and showed that only when the two lobes were in contact did an intermediate lobe-like structure appear. He suggested that the structure of the intermediate lobe and of the pars tuberalis may be due to a direct influence of the pars nervosa and infundibular body upon the pars oralis lying in contact with them. However, since in the armadillo, chicken (DeLawder, Tarr and Geiling, '34), whale and manatee, the intermediate lobe substance can be demonstrated in anterior lobe extracts, and since a discrete intermediate lobe does not exist in these animals, cells having a secretory function similar to that of the intermediate lobe cells of other species are evidently present in the anterior lobe of these animals. These cells are probably scattered among the anterior lobe cells and are indistinguishable from them by the staining methods employed.

There is no obvious phylogenetic significance to the absence of a pars intermedia. While this lobe is apparently lacking in all birds, it is well developed in the reptiles (de Beer, '26). Although all Cetacea examined have no intermediate lobe, it is present in two other members of the Edentata, namely the sloth and the anteater (Wislocki, '38). It is also present in the Marsupialia (Parker, '17). Aquatic mammals of higher orders such as the seal (Wislocki and Geiling, '36) and the walrus (Oldham, unpublished observations [6]) have a well-developed intermediate lobe.

The low oxytocic content of the armadillo hypophysis is of considerable interest. A similar discrepancy between oxytocic and pressor content has been found in the glands of the finback, blue, humpback and sperm whales (Geiling, '35; McClosky, Miller and LeMessurier, '36). In the case of the whale, however, the glands could not be removed until 8 to 24 hours after the death of the animals, so that a postmortem loss of activity is possible. On the other hand, the armadillo glands were fixed immediately after the death of the animal. The difference in hormone content indicates that in this species, the oxytocic and pressor principles are separate entities. This is in contrast with the reports of Simon ('34) and Simon and Kardos ('34) in the hypophyses of the rat, cat, guinea pig and rabbit. In these animals, the oxytocic and pressor contents were found to run parallel. Furthermore, if the animals were kept on a water-free diet, there was a decrease in both principles.

The absence of appreciable amounts of the melanophore-dispersing hormone in 'stalk' extracts shows that the pars tuberalis, which is extensively developed in the armadillo, has not assumed the function of the intermediate lobe in this animal. The presence of both oxytocic and pressor activity is to be expected in view of the similar histological structure of the neural stalk and neural lobe.

The large colloid-filled clefts seen in the posterior portion of the anterior lobe may possibly be remnants of Rathke's pouch. Similar clefts have been reported in various other

[6] This gland was kindly supplied by Dr. George Crile of the Cleveland Clinics.

animals and are considered by some as being pinched off diverticula of the pouch. Kirkman ('38), in a study of the guinea pig hypophysis, considers these have a physiological rather than an embryological significance. It is felt that in the armadillo their location and the constancy of their appearance indicate that they are remnants of the pouch.

The alpha and chromophobe cells of the anterior lobe of the armadillo hypophysis correspond closely to similar cell types in other species. The beta cells, however, appear in limited numbers and because of their deeply-staining, irregularly-shaped nuclei and frequently vacuolated cytoplasm, they are suggestive of degenerating cells. With the techniques employed, neither type of granular cell stains very satisfactorily and it is possible that cells considered here as chromophobes are actually finely granular beta cells. Bryant ('30) reported a similar difficulty in distinguishing between these two types in the rabbit hypophysis, and Smith and McDowell ('30) were unable to demonstrate basophils satisfactorily in the hypophysis of the mouse.

The large cells seen in the anterior lobe of the armadillo hypophysis have apparently not been described in other species, although a somewhat similar cell was seen in the anterior lobe of the manatee hypophysis. However, in the case of the manatee, only one gland was available for study and this was not fixed until some 5 hours after the death of the animal. Wolfe, Bryan and Wright ('38) mention that large cells appear frequently in old rats. The large cells in the armadillo hypophysis were seen in the day-old specimen and in two animals in which the sphenoid bone was not completely calcified. The constancy of their distribution and the absence of intermediate forms speaks against them being merely swollen granular cells. They may represent an additional type of epithelial cell whose functional significance is as yet unknown. Although their large size and large vesicular nuclei are suggestive of sympathetic ganglion cells, neurofibrils have not been demonstrated. In some of the hematoxylin-eosin-azure II preparations, large azure-staining masses can be seen which

are somewhat suggestive of Nissl substance. Elucidation of the nature of these cells requires a more detailed study of their cytology and embryogenesis.

Two types of cells are seen in significant number in the neural lobe of the embryo and adult armadillo. The cells with a small nucleus and long cytoplasmic processes are similar to those described by Bucy ('30) in the ox and called by him 'pituicytes.' Similar cells have been shown to exist in the neural lobe of many other species. They are considered by Bucy to be a special type of glial cell. The second type of cell may possibly be merely 'pituicytes' in a different stage of activity from those with the smaller nuclei. An alternative suggestion is that they are neuroblastic derivatives of the ependyma. The large vesicular nuclei and the cytoplasm staining deeply in some of the cells suggest somewhat the appearance of a nerve cell.

Gersh has recently described 'glandular' cells in the posterior lobe of many species. These cells have long cytoplasmic processes and contain granules which may be seen in the fresh tissue or in material fixed in Maximow's fluid and post-osmicated. He described changes in these cells during certain physiological conditions. While his methods have not been applied to the armadillo, the neuroglia-like cells correspond closely to Gersh's glandular cells and may be identical with them.

This work was carried out under the direction of Dr. E. M. K. Geiling of the department of pharmacology and Dr. William Bloom of the department of anatomy, whose helpful suggestions and criticism have been greatly appreciated.

CONCLUSIONS

The distinctive microscopic feature of the armadillo hypophysis is the absence of a discrete intermediate lobe. The pars buccalis is completely separated from the pars nervosa by connective tissue and no pars buccalis cells can be seen in the pars nervosa.

Chromophobe and chromophil cells similar to those of other species are found in the anterior lobe of the armadillo. In addition, a large type of cell is present. Two main types of cells are present in the neural lobe. One is a neuroglia-like cell similar to those described in the neural lobe of other species. The nature of the second type of cell is not known.

The embryological findings indicate that in the armadillo the absence of a discrete intermediate lobe is not due to early closure of Rathke's pouch.

The melanophore-dispersing hormone is present in the anterior lobe extracts. Pressor, anti-diuretic and oxytocic hormones are present in neural lobe extracts. Traces of all four hormones are present in the 'stalk.'

The pressor and anti-diuretic activities of the neural lobe of the armadillo hypophysis are present in the same concentration as in the standard powder. The oxytocic content of the armadillo hypophysis is, however, only about 10% that of the standard powder.

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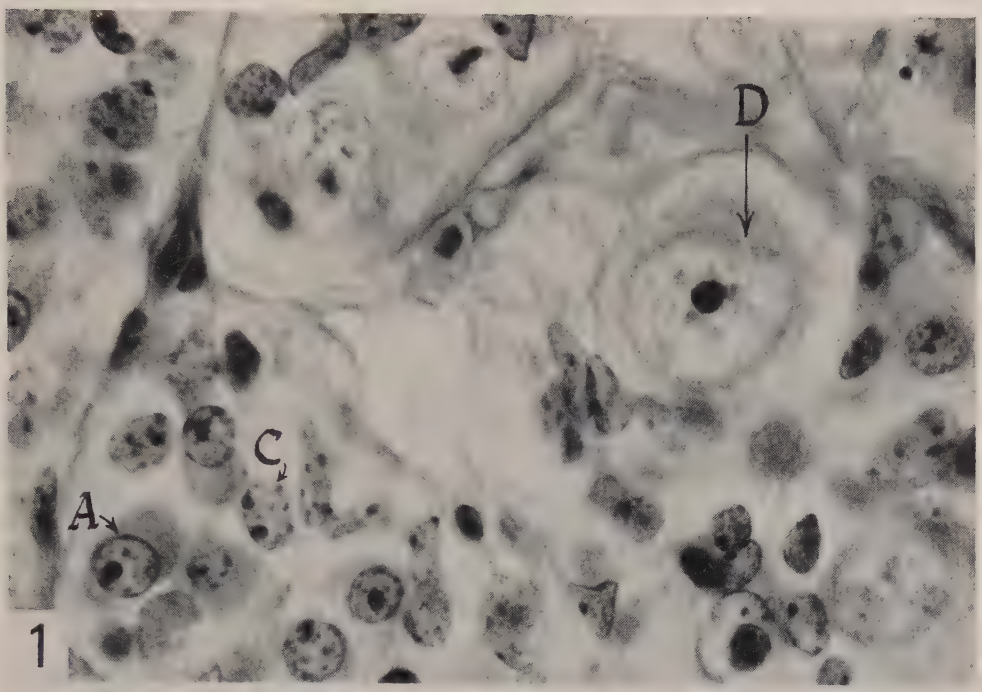
PLATE 1

EXPLANATION OF FIGURES

1 Armadillo hypophysis: anterior lobe, Zenker-formol, Mallory-azan. × 1225. D, large cell; A, acidophil cell; C, chromophobe cell.

2 Armadillo hypophysis: neural lobe, Zenker-formol, Mallory-azan. × 1225. A, neuroglia-like cell; B, group of cells with large vesicular nuclei.


D
C
A
1

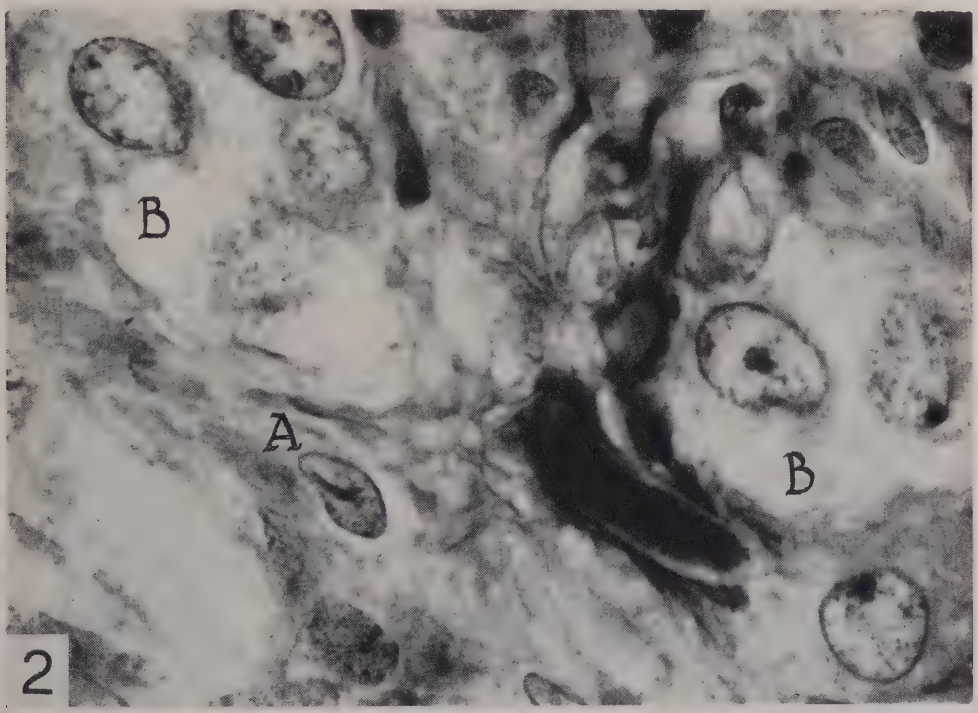
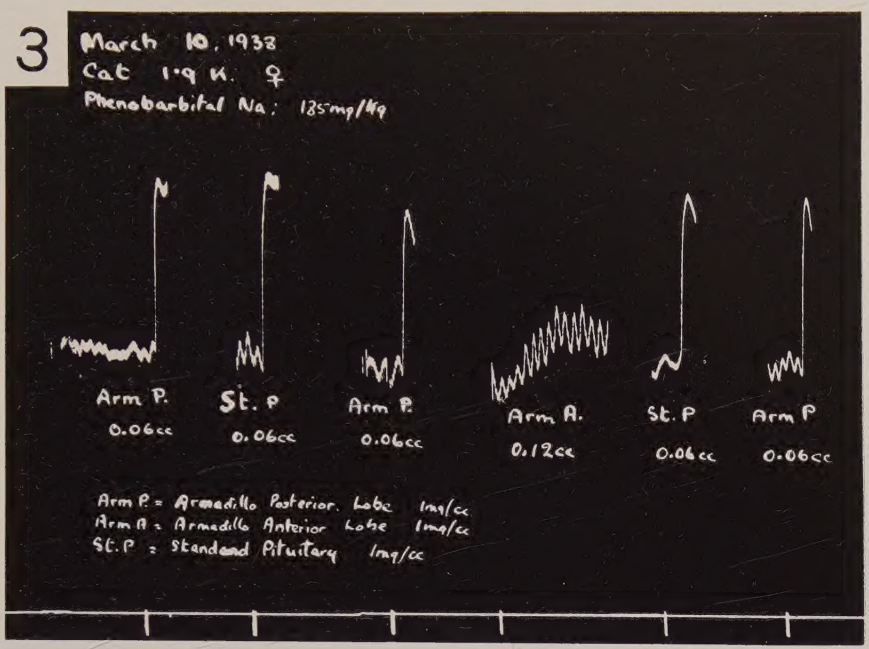

B
A
B
2

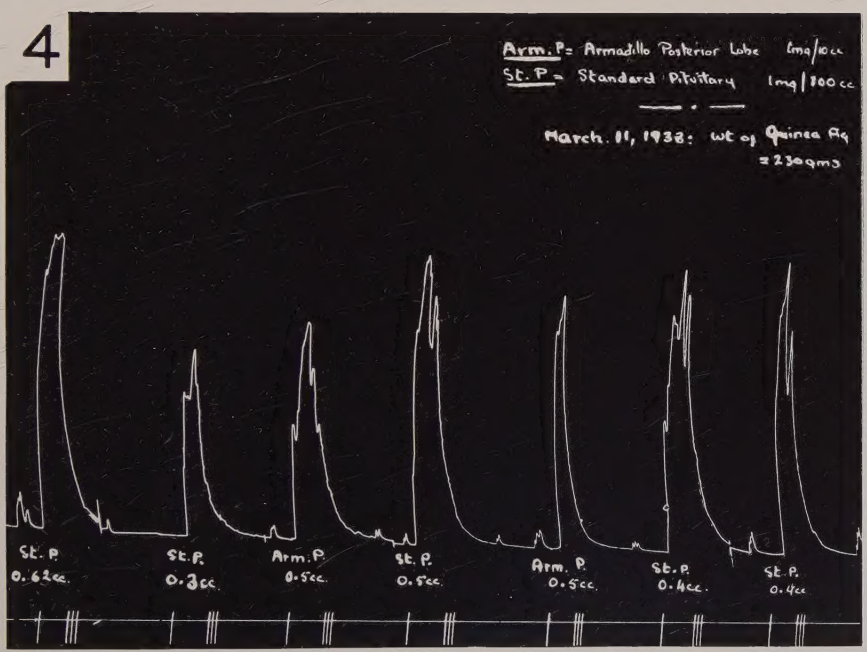
PLATE 2

EXPLANATION OF FIGURES

3 Blood pressure tracing; female cat 1.9 kg., sodium phenobarbital anesthesia, injections at approximately 30-minute intervals.

4 Contractions of uterus of virgin guinea pig, 230 gm. Arm. P, armadillo neural lobe, 1 mg. in 10 cc. distilled water; St.P., standard pituitary, 1 mg. in 100 cc. distilled water.


3
March 10, 1938
Cat 1·9 K. ♀
Phenobarbital Na: 125 mg/kg
Arm P.
0.06cc
St. P
0.06cc
Arm P.
0.06cc
Arm A.
0.12cc
St. P
0.06cc
Arm P
0.06cc
Arm P = Armadillo Posterior Lobe 1mg/cc
Arm A = Armadillo Anterior Lobe 1mg/cc
St. P = Standard Pituitary 1mg/cc


4
Arm. P = Armadillo Posterior Lobe 1mg/10cc
St. P = Standard Pituitary 1mg/100cc
March. 11, 1938: wt of Guinea Pig
= 230gms
St. P
0.6cc.
St. P.
0.3cc
Arm. P.
0.5cc.
St. P.
0.5cc.
Arm. P.
0.5cc.
St. P.
0.4cc.
St. P.
0.4cc